Analysis of Biological Molecules by Single Photon Ionization

Praneeth Edirisinghe,[a,b] Jerry Moore,[a,c] Wallis Calaway,[a] Igor Veryovkin,[a] and Michael Pellin,[a]
Kelly Skinner-Nemec,[d] Carol Giometti,[d] and Luke Hanley[b]

[a] Materials Science Division, Argonne National Laboratory, [b] University of Illinois, Chicago,
[c] Current affiliation: MassThink, Naperville, IL, [d] Biology Division, Argonne National Laboratory

MOTIVATION: To identify biological molecules on surfaces and in complex mixtures.

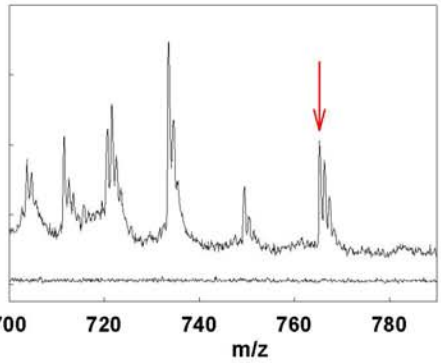
- Surface-bound peptides, proteins and other biomolecules are used for microarrays, microfluidic channels, cell growth surfaces, and biosensors.
- Mass spectrometric detection of surface bound biological molecules and molecules within biofilms is difficult by conventional means (e.g., MALDI).
- Separating the desorption and ionization steps as is possible with laser post ionization of desorbed neutral molecules can improve detection.
  - By increasing sensitivity.
  - By decreasing molecular fragmentation.
  - By increasing spatial resolution.
- Single photon ionization (SPI) is promising for soft ionization, i.e., high ionization yields with minimal fragmentation of large molecules.
  - F_2 lasers are intense sources of vacuum ultraviolet (VUV) light, but the photon energy (7.9 eV) is too low for SPI of many biological species.
  - Photo ionization tags can be selectively and covalently attached to the analyte of interest allowing SPI of most species.

MAJOR ACCOMPLISHMENTS: Chemical tagging coupled with VUV SPI allows sensitive detection of intact species.

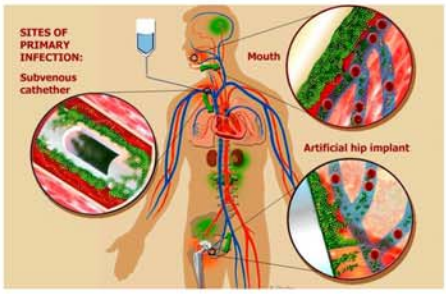
- Chemically tagging methods were developed for large biomolecules, such as peptides, allowing efficient SPI using 7.9 eV photons.
  - By selective derivatization, high sensitivity and low backgrounds were achieved, allowing peptides to be detected at low concentrations.
  - Specific tags were shown to help stabilize photoions thereby permitting detection of intact molecules.
  - Peptides up to mass 3100 were observed without fragmentation.
- The technique allows detection of quorum sensing peptides in biofilms.
- Laser desorption was found to be optimal for chemisorbed species, while ion sputtering gave the best signal for physisorbed biomolecules.

IMPACT and FUTURE DIRECTIONS

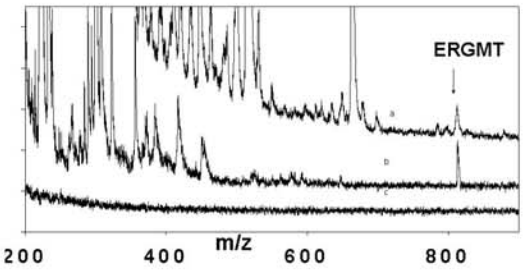
- VUV tagging has broad applicability to the study of organic and biomaterials.
- Uses include probing bio-material interfaces and medical diagnostics.
- Proteins and polysaccharides are being studied to extend the technique to larger molecules that have a tertiary structure.
- The study quorum sensing peptides in biofilms will be extended to imaging of peptides in a complex biological mixture.
- Studies of the photoionization mechanism are planned to verify direct photoionization (rather than droplet-vaporization or intramolecular charge transfer and dissociation).



The upper curve above is the time-of-flight mass spectrum of the tagged peptide, GAPKSC, laser desorbed and post ionized by SPI. The intact tagged peptide (indicated by the red arrow) is seen only when both the desorption laser and the photoionization laser are present. For an untagged sample, no signal above 700 Daltons is detected.



Biofilms, which naturally form over artificial implants, are sites of primary infections. These infections are difficult to study in situ since biofilms masks the quorum sensing molecules.



The above laser desorbed post ionized mass spectra show that an anthracene tag and SPI allow the quorum sensing peptide, ERGMT, to be detected within a biofilm. The upper two curves (a & b) are of *Bacillus Subtilis* with and without a biofilm, respectively. Both show a pronounced signal at the anthracene-tagged ERGMT peptide mass. The lower curve (c) which is the same *Bacillus Subtilis* biofilm without derivatization shows no discernable mass signals.

P. D. Edirisinghe, S. S. Lateef, C. A. Crot, L. Hanley, M. J. Pellin, W. F. Calaway, J. F. Moore, Analytical Chemistry 76 (2004) 4267-4270.

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